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CONSIDERATIONS REGARDING THE REDUCTION OF TETRAZOLIUM SALTS (A REVIEW)

by J. Verne and R. Wegmann.

Introduction.

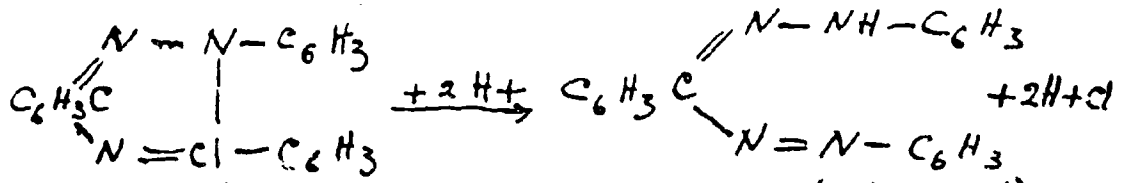
Tetrazolium salts play a preponderant part during the transfer of hydrogen in the chain of enzymatic reactions which lead to the reduction of a substance by oxidation of another. Their use is becoming general, but, simultaneously, there is an expansion of the category of substances capable of reducing tetrazolium salts. We thought it worthwhile to re-examine the question and to draw some useful conclusions.

The most frequent use of tetrazolium (TZ) derivatives is in connection with the detection of tissual dehydrogenases. They serve, in the same way as methylene blue, as acceptors of hydrogen and function as redox indicators. The reduction of methylene blue yields a colorless substance, the leuco-blue, the speed of whose formation measures the rate of dehydrogenasic activity. That reduction is reversible. Furthermore, it requires very strict anaerobic conditions. The TZ's, which are colorless and soluble in water, bring about, through reduction, a formation of colored derivatives, the formazans, which are not soluble in water. This process is irreversible and does not require the absence of oxygen.

Therefore TZ salts present an undeniable advantage over the use of methylene blue, for they make possible a precise localization of the point of formation of the formazans (FZ), both in the tissue and in the cells. The clearness and intensity of the reaction depend in particular on the variety of the TZ's which are used, on the specific activity of the tissue, but also on a number of factors which we shall examine later, such as pH, isotonicity, presence of cations, a well-plugged medium, duration of incubation, not to mention the mode of manipulation of the tissue itself. All those conditions, connected with the choice of the substrate, combine to delimit the nature of the substances which bring about a reduction of TZ salts.

1. Different tetrazolium salts.

There are several tetrazolium salts which all possess characteristics enabling them, to a various degree, to achieve the histochemical detection, or to serve in biochemical dosages. The first to be utilized was the 2-3-5 triphenyltetrazolium or TTZ (Kuhn and Jerchel, 1941) (28). Its general constitution and its reduction to FZ is represented by fig. 1.



Triphenyl salt (colorless) tetrazolium or TTZ

(red color) Formozan (color : red)

Figure 1

There are, furthermore:

- 2-3 diphenyl 5 methyl tetrazolium (40) chloride or DTZ.
- 2-2' (p-diphenylene)-bis-(3-5 diphenyl-tetrazolium) chloride, which is Neotetrazolium or NTZ.
-- 3:3'-diamisole bis 4:4' (3:5 diphenyl-tetrazolium) chloride, or tetrazolium Blue (43) or BTZ. It is therefore the dimethoxy derivate of neotetrazolium.
- 2B neotetrazolium (49) phosphate or PTZ.

2.5 diphenyl 3 (4-styrylphenyl) tetrazolium chloride or M and B 1767 (43), or STZ.

--2(p-iodophenyl) 3 (p-nitrophenyl) 5-phenyltetrazolium (54) chloride, or ITZ.

-- violet tetrazolium, whose formula is unknown (56), VTZ.

FTZ yields a red formazan, BTZ a blue FZ, and so dies NTZ. Pearce (43) has noted that BTZ yields FZ's of a different color, according to the compound: blue to purple for the sulfhydrides, red for the lipids, blue for sugar reducing agents. The reduction of TZ salts is achieved progressively (Jerchel, 1949) (26) and forms a red semi-reduced compound, then a reduced blue compound (Kun, 1951) (29).

According to the studies made by Shelton and Schneider in 1952 (56) regarding the differences in the activity of TTZ, BTZ, NTZ and VTZ, and, according to the comparative study concerning TTZ and NTZ made by Cascarano and Zweifach in 1955 (10), TTZ is inferior to NTZ when it comes to obtain cytological details. It is spontaneously reduced, pales under the impact of light, or with the passing of time. It will not be reduced by the blood vessels, the skeletal muscles and the peripherical nerves (10). For Shelton and Schneider (56), BTZ appears inferior to NTZ because of the long period of incubation and of the irregularity of the precipitation of the formozans' crystals. VTZ has a major drawback: its formozans' crystals are long and fine and destroy cell stuctures (56).

The use of a TZ derivative rather than of another derivative has been prompted by those differences of reactivity. Sometimes, it was also difficult to obtain the various kinds of TZ. Their use must be considered in funtion of the problem that must be studied and it can't be stated that one is absolutely superior to the

other.

TTZ is thus used by Black and his aides (4,5,6,7,64), by Brodie and Gots (9), by Bodine, Lu and West (8), by Cascarano and Zweifach (19), by Fried and Zweifach (20), by Gomori (21), by Gonse and Yotsuganagi (22), by Jensen, Sacks and Baldauski (25), by Jerchel (26), by Kuhn and Jerchel (28), by Kun (29), by Kun and Abood (30), by Lillie(31), by Lison (32), by Mattson, Jensen and Dutcher (35), by McMillan, Klatzo and Duff (37), by Everson Pearse (43, 44), by Roberts (48, 49), by Shelton and Schneider (56), by Smith (58), by Steigleder (60).

BTZ has been used much less, in particular by Cooper (11), Pearse (43), by Rutenburg, Gofstein and Seligman (52), by Findlay (16).

NTZ occupies an intermediary position and has been used especially by Bajusz and Szirmai (2), by Cascarano and Zweifach (11), byForaker and Denham (17), by Foraker, Denham and Mitchel (18), by Fried and Zweifach (20), by Martin, Cooper, Chaudhuri and Green (34), by Mustakallio (38, 39), by Mustakallio and Jannes (40), by Pearse (43, 44), by Rennels and Ruskin (46), by Shelton and Schneider (56) and by Villareal and Burgos (63), by Findlay (16).

Some other salts have been used only rarely, the ITZ by Defendi and Pearson (12) and by Pearson and Defendi (45), by Seligman, Gofstein and Rutenburg (54); DTZ by by Roberts (49), PTZ by Roberts (49). VTZ by Shelton and Schneider (56) and by Roberts (49), STZ by Pearse (43) and TZ purple (PPTZ) by Ortmann (42) and by Steigleder (61).

Table 1 provides a summary of the results obtained by some authors who have used several TZ salts and compared the results.

Author	Ref.	TTZ	BTZ	NTZ	VTZ	PTZ	DTZ
Cascarano	(10)	2	—	3	—	—	—
Findlay	(16)	2	2	3	—	—	—
Pearse	(43)	—	2	3	—	—	—
Roberts	(49)	1	2	3	2	2	0
Shelton	(56)	2	3	3	3	—	—

TABLE 1

Mean intensity of reactions by various TZ salts.

(o : no reaction; 1 : feeble reduction; 2: mean reduction; 3:strong reaction).

2. Nature of the compounds reduced by TZ.

If TZ salts serve as activity tests of a whole series of compounds characterized by their reducing power, the nature of those substances can be very variable.

a) Endogenous reductases.

The primary condition for bringing them into evidence is the use of fresh cuts, which have not been frozen, nor dried, nor, especially, fixed in a fixator of any nature whatsoever (Shelton and Schneider (56)). The simple section of cuts with a rezor may damage them (Cascarano and Zweifach (10)), and so may also do the contact between them and the water which collects the cuts (Seligman and Rutenburg (55)). Contact with heavy metals (Barnet and Seligman (3)) or the presence of metabolical inhibitors (Black, Kleiner and Speer (5)) destroys or prevents their action. Reduction only occurs within pH limits comprized within pH 6 and 7. It is therefore quite improbable that in those conditions it should not be the case of one or several enzymatic systems. The activity of this endogenous reductase can be intensified in the vessels by addition of mannose, which appears to act rather as a co-factor rather than in a role of substrate (Reid and Zeifach) (20).

This endogenous redactose has been sought in the kidneys (Cascarano and Zweifach) (10), (Black and Speer) (6); in the suprarenal (Black and Speer) (6); on the Ciliated (Fauré-Fremiet and Gauchery) (15); in [redacted] sea-urchin eggs (Gonse and Yotsuganagi) (22); on atheromatose scales of the rabbit's aorta (Foraker, Denham and Mitchell)18); in the mesenteric capillaries and in the carotide (Fried and Zweifach) (20); in the heart muscle (Cooper) (11).

Most of those authors note an intense activity essentially localized in the lipidical enclaves. It is intense in the glomerulaea and in the reticulated (?) of the mouse (Black and Speer) (6) and in the lipidical intra-atheromatose droplets (McMillan and colleagues) (37). Cooper does not detect any activity in the ventricle or the auricle (11), before and after birth, in the rat.

The precise significance of the reductasic activity is not exactly known as yet. Rutenburg, Gofstein and Seligman (52), as well as Foraker, Denham and Mitchel (18) admit that it can be superimposed to the succinodehydrogenasical activity. Nevertheless those latter authors (18) consider the endogenous activity to be twice more intense than that of succinodehydrogenase, that being at the level of the ovaries.

b) Specific reductases.

They are much easier to study, for their detection may be achieved on frozen tissue. In order to search for them, one adds a specified substrate to their incubation medium. On the other hand, it requires a well stoppered medium

Succinodehydrogenase. (SD)

The segments by freezing are incubated in a stoppered medium containing sodium succinate and a TZ salt, often with a fixation with formol following. Bajusz and Szirmai (2) study the enzyme in the muscle, the liver and the kidneys of rats after those organs have been treated with aldosterone-electrocortine. The conjunctive tissue, which had been negative in the control samples, becomes very strongly positive after injection of this steroidal hormone. Among rats the mercurial diuretics bring about a marked inhibition of the kidney SD, especially in the twisted proximal tube, which may be related to an increased diuresis. The adjunction of BAL restores the normal aspect (Rennels and Ruskin) (46).

In the kidney of rats who had been rendered hypertensive a dose of DOCA and by a salted fare, no change of the reaction, which is normally intense, occurs (Eranko and Niemi) (14). Infection with Mycobacterium tuberculosis provokes in the guinea-pig a marked fall of renal SD. The adjunction in vitro of a tissue factor extracted from the kidney of a normal ox and with a structure of a nucleotide associated to a sulfhydrile, restores normal activity (Marten, Cooper, Chaudhuri and Green) (34).

Cooper (11), on pre- and post-natal rat hearts, notes an increase of the SD activity, which seems to him to parallel the increase in the number of mitochondries. In the ovaries, a strong SD activity is observed in the folliculous cells and in the stroma cells. It is very marked in the lutenic cells and has a small intensity in the hile vessels. Foraker, Denham and Mitchel conclude that there is, eventually, a correlation between the strong SD activity and the hormone production; Ortmann arrives at a similar conclusion (42) in his study on the placenta. The cytotrophoblaste, which is supposed to be an important area of hormone production, is very rich in SD. It is the same case with the works of Meyer, McShan and Erway (36), on the subject of the ovary and the lutenic cells.

Mustakallio (38) has made an interesting observation on the epithelium of the gall bladder and in the liver of a pregnant mouse. The SD activity, strongly marked in the non gestating mouse, diminishes in very strong proportions under the impact of the pregnancy, and seems to be only slightly influenced by fast or by ingestion of food. This author attributes this fall in the SD rate to an increase in the ostrogens rate.

In the malignant hepatic tumors, induced by p-dimethylazobenzene, Pearson and Defendi (45) do not note any constant differences of SD activity between the normal cells and the tumoral ones. It is easy to understand the importance of the study of SD in the neoplasical processes (Rutenburg, Gofstein and Seligman) (52).

While the specificity of the succinodehydrogenasical activity can hardly be

doubted, certain authors like Fried and Zweifach (20), do not admit the presence of a specific enzyme SD in the vessels (endothelium and smooth muscle), as a result of the positive reaction obtained also with other substrates. We think that this restriction cannot be generalized for all tissues and organs. In support of this hypothesis, one can mention the works of Steigleder on the subject of the derm and the epiderm (60, 61), where there is no parallelism between the activity SD and the activity of other substrates in the pilous follicle and in the collar of the sebaceousglands.

A more important problem concerns the activity or the absence of reduction intermediaries in the chain of hydrogen transport. Kellin and Hartree (27) think that there is a BAL-sensitive factor, probably a hematine, which serves as an intermediary between the succinodehydrogenase-cytochrome b agent and Cytochrome. If there is no other factor associated with this system, one would have the right to admit a specific evidence of the existence of SD at the exact spot of the reduction of TZ. The question is not solved, as yet. Defendi and Pearson (12) rightly that methylene blue is directly reduced by succinodehydrogenase, without the intermediary of the BAL-sensitive factor.

The study of SD presents at least the advantage of a quantitative analysis perfected by Defendi and Pearson ( ). Villareal and Burgos (63), finally, in a comparative histochemical and biochemical study of SD in the gastric mucuous of the rat and of the frog, succeed in determining most clearly the relations between the Krebs cycle and the main cells. At their level the SD reaction is the most intense and it can be activated with acetyl-beta-methylcholine, or inhibited with mercurial diuretics. BAL diminishes the hyperactive reaction which is useless from the point of view of experiments.

Altogether, the use of TZ's in the study of the SD activity opens here an interesting field for research and puts this reaction among the most interesting enzymatic histochemical reactions.

β-glycerophosphodehydrase (GPD).

It has been studied by Steigleder in the skin (60) and manifests itself actively in the pilous follicle and in the collect of sebaceous glands.

lactodehydrogenase (LD).

Also analysed by Steigleder (60), it is also active in the pilous follicle to the same degree as SD or GPD. On the other hand, it is almost absent in the collect of the sebaceous glands. According to the works of Strominger and Lowry (62), on the brain, the LD activity is parallel to the aldolasic activity. In order to become manifest, it requires the association of DPN (diphosphonucleotide). The absense of an addition of DNP may explain the results obtained by Steigleder.

malico- and glutomodehydrogenases (MG and GD).

In principle, these enzymatic systems can also be studied, since the research carried out by Strominger and Lowry (62), which was histochemical and qualitative, at the brain level, has shown clearly marked differences of activity between these various enzymes, probably in connection with metabolical differences. Furthermore, these enzymes are poorly thermolabile .

desulfhydrase or desulfurase (DS)

Its detection presents numerous problems, which are far from being solved. For, if one adds some cystein or some gluthation reduced to an incubation medium, one can wonder whether it is the cystein which directly reduces tetrazolium salt or if it is the desulfurase which acts on the cystein. In vitro, cystein reduces TTZ, PTZ and NTZ, in growing order (Roberts) (49). Cystein reduces none of the TZ salts. The addition of cyanide to cystine provokes, in growing order, a reduction of TTZ, of BTZ and VTZ and of NTZ. The glutathion associated with the cyanide reduces TTZ slightly and reduces strongly those other TZ salts (Roberts) (49).

All those reactions have been carried out by Roberts (49) with pH 74. Those results do not tally with those obtained by Fried and Zweifach (20), who obtained no reduction for the reduced glutathion, but , on the contrary, an inhibition, in the mesovaric vessels and in the carotide. Smith (58), when pH is above pH 9, obtains an increased reduction by cystein. One cannot, therefore, take into consideration all those reductions obtained with pH below 9; in any case, the doubt regarding the presence of desulfurase remains, because of the spontaneous reduction by sulfhydrile groups in vitro. Barrnett (sic) and Seligman (3) similarly found a reduction of the TZ blue with disulfide groups reduced in vivo with cyanide. While it is impossible to dissociate the activity proper to exogenous sulfhydriles from the enzymatic reduction with DS, there is nevertheless an indirect way to evaluate the DS activity. It consists in adding cyanide to the incubation medium, that cyanide having a pH above 9. The cyanide reduces the disulfide functions which are present and forms sulfhydriles, which teduce the TZ salts. This DS activity cannot be evidenced except on fresh material, or at least material which has not been included with paraffin. The results reported by Everson Pearse (43), nevertheless, relate solely organs fixed with formol or with a formol-sublimate, where the desulfurases have been destroyed. Nevertheless, according to observations made by Pearse (43), the strongly alcaline medium extracts, among other matters, proteins and muco-proteins. It does not seem that, up to now, it has been possible to study directly tissular desulfurases, in spite of their obvious interest. Lillie (31) admits, however, that the addition of cystein makes it possible

to study the desulfhydrasis.

Direct reduction by means of reducing agents.

As we have just seen, substances with sulfhydrile functions, when added to sections in incubation with TZ, reduce it directly. If it is true that the presence of these sulfhydriles stabilizes in vitro the reduction of NTZ (Findlay) (16), the addition of a single cyanide can already provoke a reduction of TZ. The association of cyanide and of cystein considerably increases the quantity of formazan which is produced (Findlay) (16).

In fact, the reduction of the TZs is achieved not on fresh sections (cuts), but on cuts of organs fixed in various fixators, such as acetone, neutral formol, or preferably trichloracetic acid in 80% alcohol (Findlay) (16). Then the groups -- SH and SS -- present in the tissue are characterized. Everson Pearse recommends formolated fixators (43). The cuts are cut during the freezing (16, 43) or are even used after iclusion of paraffin (43). While Pearse recommends a pH of 12.8, Findlay recommends a pH 10, having noticed a very weak reactivity of cystein to pHs above 11. On the contrary, glucids react favorably to pHs above 11. On the contrary, glucids react favorably to pH's above 11 (Smith) (58); Findlay (16).

The presence of sulfhydrile groups has been put in evidence by Findlay (16), by Pearse (43) and by Steigleder (60), mostly in the epiderm, in the proteins(?) and in microproteins, as well as in the hairs of the phaneres (Rogers) (50). Bodine, Lu and West (8) also admit a TZ reduction by sulfhydrile groups, which apparently is produced in the cells in mitosis, and in Penicillium chrysogonium, according to Fred (sic) and Knight (19). In the meristeme of plants this reduction by means of sulfhydrile groups is especially marked (Roberts) (49).

An addition of cyanide makes possible the formation of sulfhydrile groups on the basis of disulfuric groups, at the rate of two groups -- SH per group -- SS -- (Findlay (16). An inhibition of sulfhydrile groups is achieved by means of iodocerate. A better scission is achieved by means of iodacetate is achieved by means of thioglycolate (Barnett and Seligman (31) and Findlay (16). A blocking of all groups -- SH thus firstly requires a blocking of the groups -- free SHs, followed by a scission of the SS groups which are also blocked .

Beside the preceding compounds, other reducing substances may intervene, such as ascorbic acid. For Defendi and Pearson, in vitro, (12), ascorbic acid reduces triphenyltetrazolium, with pH at 9.1. On the contrary, Mattson notes no reduction with this same pH'(35). Kuhn and Jerchel (28) had already proven that this substance cannot be made responsible for the reduction of the TZ salts, for it does not occur above pH 9. If one excludes the possibility of endogenous reduction

with ascorbic acid, one can achieve its exogenous reduction. Steigleder (60) has applied it to the study of keratine and notes a reduction in the keratinized zones.

Can glucids bring about a reduction of TZ salts? According to Jensen and his colleagues (25), reducing sugars do not reduce TZs below pH 11. Pearse (43) obtained, with pH 12.6, the reduction of a whole series of reducing glucids, in particular with glucopyranose monomers. Polymers react less favorably, as in the case of glycogen. Findlay obtains a reduction of glucose at pH 10.2, while Smith (58) does not notice any reaction below pH 11. It must be noted that in the test of Findlay (16) reduction was achieved in the presence of cyanide, whose reducing power on the TZs is known. Glucogen has yielded doubtful results. The reduction at the level of the vessels studied by Fried and Zweifach (20) leads those authors to admit a cofactor role on the part of glucids, and more precisely on the part of mannose. Glucose seems to act less well, other hexoses rather little and the diascharids not at all. They do not admit, however, a direct reduction by those glucids. The pH of the middle part of the incubation is only of pH 7.3, and cannot promote a reduction.

The reduction of other substances has been considered. Pearse (43) gets in vitro a reduction of the DOPA. Black and Speer (6) reduce it with cortisone, with F Compound and with DOCA.

According to all those data, it will be noted that the FZs can derive a whole group of reducing substances, but it is the addition of those substances to the cuts which will condition the response to be obtained. On organs in which the enzyms have been destroyed by fixation, the number of reducing compounds must become rather small and will be limited by the pH. If the pH is inferior to 11 but above 9, one can study mainly the sulfhydrile and disulfide groups. With pH in the neighborhood of 13, one will rather get reducing sugars. The analysis table presented by Pearse (43) must be completed with an indication of the pH.

3 - RELATION WITH THE LIPIDS

Lipids have a particular affinity with the derivatives from the reduction of TZs. They never contain FZ crystals and always take a pink or red coloration, with TTZ as well as with BTZ and NTZ. The grains of lipofuscines on the cuts included with paraffin take those colorings (Pearse) (43). In the srrenals, the coloration occurs in the lippidic enclaves in the zones which secrete steroid hormones. The coloration is restricted to those enclaves and serves as their cortico-surrenal functioning index. For Edwards and Ball (13), the phospholipids have a role of cement and of vector between the various carriers of hydrogen of the succino-oxydasical complex.

Is this a case of a special affinity of the FZs for the lipids? (Gonse and Yotsu-

ganagi) (22). Or the case of a diffusion of the FZs formed by other substances towards the lipids? Steigleder notes a close parallelism between NADI and the FZs. Yet it seems that, under the impact of aldosterone and DOCA in a suuenalectomized animal, it is no longer the enclaves which are reacting, but the lipoproteic base (Bajusz and Szirmai) (2).

The problem thus remains as to whether the enclaves directly reduce the salts of TZ.

4- RELATIONS WITH MITOCHONDRIES.

The specific succinohydrogenasical activity is very high in the granular fraction of the testicle obtained by Greif (23), where it is associated with the hyaluronidasical activity. It is parallel to the increase in the number of mitochondries in the gall bladder (Mustakallio) (38) as well as in the heart (Cooper) (11). The reductior did not occur at their level, but in their vicinity. The coloration of the mitochondries appears to be only secundary. When the FZ crystals occur, it is always outside the mitochondries. At that moment the coloration of the mitochondries disappears. In the presence of lipids no grain coloration occurs. Only the fat enclaves are tainted (Gonse and Yotsuganagi) (22). Harman (24) confirms this enzymatic activity.

If there actually is an SD activity at their level, it does not explain in which other elements one must seek the remaining 65% of SD activity.

5- PHYSIOLOGICAL ROLE.

Surrenalectomy diminishes in all organs the reaction to NTZ (Bajusz and Szirmai) (2). DOCA inhibits the SD activity of the glomerular (gland), without touching other zones (Zweifach, Black and Speer) (64). Cortisone inhibits the activity of the glomerular (gland) (Black and Speer (6). On the contrary, on an animal carrying a tumor, the glucorticoids inhibit and DOCA stimulates the glomerular (gland) (Black and Speer) (6). While the electrocortine does not modify the distribution of the FZ in the animals serving as control samples, it brings back to normal the aspect of the organs of surrenalectomized animals (Bajusz and Szirmai) (2).

6- TECHNICAL FACTORS.

The thickness of the cuts plays an important part. Too thin cuts may give no reduction. The latter may be spontaneous on too thick cuts. Defendi and Pearson (12) find a 5 μ thickness to be sufficient for a quantitative analysis of the SD. Inversely, Cascarano and Zweifach make 1 mm cuts before obtaining a reaction. On the average, a thickness of 20 to 30 μ seems to be right.

An incubation duration of 30 to 60' intensifies the reaction of the surrenal (gland). In the kidney, on the contrary, it brings about an aberrant modification of the crystals and a variation in their shape (Cascarano and Zweifach) (10). An average duration varies between 20 and 60 minutes. It also depends on the salt which is employed and on the aim which is to be achieved. For a quantitative dosage 5 minutes are sufficient (Defendi and Pearson) (12). For SD between 30 and 120 minutes are necessary, for the sulfhydrile groups between 40 minutes at 60° C (Pearse) (43) and 4 hours at 37°C (Findlay) (16).

For the study of enzymatic systems, tensio-active substances must be rejected, for they suppress the coloration of the FZ. On the contrary, for Findlay (16), an addition of Tween 40 increases the reactivity.

An addition of inhibitors to the incubation media prevents the formation of FZ. Cyanide, azide, malonate inhibit the reduction adypocytes, but not in the vessels (Fried and Zweifach) (20). In the study of endogenous reductases, a simple agitation during the incubation (Gonse and Yotsuganagi) (22), intempestive aeration (Fried and Zweifach) (20), darkness (22) may prevent the reduction. for the study of the -SH functions, the iodacetamide, the iodacetate play the part of inhibitors. Preferably, the use of ether for the killing must be avoided (Malaty and Bourne) (33).

The activators are the glucids (see above), light (22) and, in principle but not in fact, the DPN. (20, the Al, Mg ions, the carbonates (Rutenburg, Wolman and Seligman)(53), the Ca and Al ions (Keilin and Hartree) (27), very stric anaerobic conditions (53). A well stoppered medium, to which ions of Cl, Ca, PO_4 and Na have been added, provides better results (Fried and Zweifach) (20). That is how Cascarano and Zweifach had noted the presence of extracellular depositions in the absence of electrolytes of the incubation medium.

7- LIVING COLORATION.

Tetrazolium salts have also been used for that purpose (35 and 47).

CONCLUSION

This brief review of the reduction of TTZ salts shows that numerous factors intervene in the course of their application to the study of various substrates and that it is not always easy to compare the results because of the diversity of the techniques which have been used.

SUMMARY

Tetrazolium salts are reduced by various substances. On fresh, non-frozen cuts it is possible to put in evidence the endogenous reduction. On cuts obtained by congelation or frigodessication, this reductasis is destroyed. The addition of

a substrate, such as succinic acid, brings int- evidence the succinohydrogenasis. Other substrates may be used, such as substances with sulfhydrile functions: then sulfhydroses occur.

On fixed cuts, included or not with paraffin, at pH 10, one detects, in principle, the sulfhydrile functions. With pH 12.8 reducing glucides are essentially characterized. Those are endogenous substrates. Other substances may also be present and they are considered in their relations with the lipids. Finally, TT salts may serve as life colorants.

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